unbound protein, while it was not found for the ATP-bound protein. These findings suggest that binding of ATP controls the opening/closing motion responsible for a structural change from outward-facing into inward-facing, where the drug binding site is accessible from the cell interior.

3P-082 機械受容型イオンチャネルダイナミクスの統計的解析

Statistical analysis of the mechanosensitive ion channel's dynamics

Kazuto Sei (1), Chun Biu Li (2), Tamiki Komatsuzaki (2) ((1) Graduate School of Life Science, Hokkaido University; (2) Research Institute for Electronic Science, Hokkaido University)

The mechanosensitive ion channel(MscL) is a membrane protein which functions under the external force (i.e. membrane tension) by the surrounding lipid bilayer. Here we focus on the analysis of MscL dynamics. Patch clamp experiment(PCE) is one of the fundamental techniques to investigate the function of ion channels, and the data from PCE (i.e. the conductance time series) are analyzed in general by the Markov kinetic model with several states. Although this analysis provides us with the information about the free energy landscape, it is somehow difficult to adopt the result as the model of gating dynamics. There are several reasons:(1)we can not avoid specifying the number of states subjectively, (2)when the time series doesn't show the distinct conductance levels (this may be the case of MscL), it is difficult to distinguish the several levels, (3)since the fitting of the model is performed globally, the local dynamical information might be lost sometimes.

We will , therefore, take another kind of statistical approach, so-called the change point analysis(CPA). CPA finds the "change points", defined as the time when the property of the underlying system changes. In CPA, the number of states is not assumed a prior and determined by the objective criteria. We present the result of the fundamental analysis and CPA for the conductance time series of MscL dynamics, and discuss what we can learn of the gating mechanism from these analyses. (This research is collaboration with the Prof. Sokabe's group in Nagoya University.)

3P-083 バクテリオロドプシンミュータント L93A の長寿化させた光反応中間体の結晶学的研究

Crystallographic study of a long-lived photoreaction intermediate of the L93A mutant of bacteriorhodopsin

Yoshikazu Yamazaki (1), Masanori Hikake (1), Midori Murakami (1), Kunio Ihara (1), Tutomu Kouyama (1) ((1) nagoya university)

Bacteriorhodopsin (bR), a membrane protein found in the plasma membrane of *Halobacterium salinarum*, functions as a light-driven proton pump. When the all-trans isomer of bR in the light-adapted state absorbs visible light, it undergoes a reaction cycle involving five spectroscopically distinguishable intermediates (K, L, M, N and O). To understand the transport mechanism, we previously analyzed the structures of several reaction intermediates (K, L and M) of bacteriorhodopsin. We also tried to determine the structures of the intermediates occurring in the late half of the photocycle remains unsuccessful, but our trials remain unsuccessful due to difficulty in efficiently trapping these intermediates. To overcome this difficulty, we crystallized the L93A mutant, which is reported to have a very long lifetime of the O intermediate. Diffraction data showed that replacement of Leu93 with alanine creates a large cavity near the C13 methyl of retinal, in which four water molecules are accommodated. In this study, we tried to obtain structural information of the O intermediate of L93A. In a difference map that was derived from two datasets that were collected from illuminated and un-illuminated crystals, we were not able to observe any clear densities. This seems to reflect a very low efficiency of formation of the O intermediate in the crystal. We are now searching for optimal crystallization conditions for investigation of light-induced structural changes in the L93A mutant.

3P-084 紫膜バクテリオロドプシンの光退色現象におけるタンパク質-脂質間相互作用の影響

Effect of protein-lipid interaction on irreversible photobleaching of bacteriorhodopsin in purple membrane

Tasuke Narishige (1), **Yasunori Yokoyama** (1), Masashi Sonoyama (1), Shigeki Mitaku (1) ((1) Department of Applied Physics, Graduate School of Engineering, Nagoya University)

Bacteriorhodopsin (bR), which is a light-driven proton pump of *Halobacterium salinarum*, forms a two-dimensional (2D) crystal together with lipid molecules, known as purple membrane (PM). We previously revealed that bR underwent an irreversible photobleaching at high temperature, in which the recovering process of photocycle is hindered eliminating the function. A kinetic study for the photobleaching showed that two kinds of bR species with respect to the structural stability existed in PM at high temperature and that a fraction of less stable species increased with the 2D-crystal melting. This strongly suggests that intermolecular interaction in PM affects the heterogeneous stability against photobleaching. Here, we studied the effect of lipid molecules on the 2D-crystal melting and the functional activity by using delipidated bR. We prepared delipidated bR using a detergent of 3-[(cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS). The results showed that delipidation caused bR destabilization under illumination at temperature range between 60 and 85 °C at

pH 7. Kinetic analysis showed that this destabilization was mainly responsible for an increase of the rate constant of slow-decayed component. We also conducted the same experiments for native PM in the presence of NaCl at high concentration and found that the similar characteristics of photobleaching as delipidated bR were exhibited at above NaCl concentration of 1 M. The effects of lipid removal and an electrostatic shielding on PM will be discussed based on these results.

3P-085 DMPC、DPPC 二層膜に再構成したバクテリオロドプシンの光退色

Photobleaching of bacteriorhodopsin reconstituted in DMPC and DPPC bilayer.

Taku Kitoh (1), Lumi Negishi (1), Yokoyama Yasunori (1), Masashi Sonoyama (1), Shigeki Mitaku (1) ((1) Department of Applied Physics, Graduate school of engineering, Nagoya University; (2) Department of Applied Chemistry and Chemical Biology, Gunma University)

Reconstituted bacteriorhodopsin (bR) in dimyristoylphosphatidylcholine (DMPC) lipid membrane was previously studied in view of the relationship between the lipid bilayer transition and the irreversible photobleaching of bR. The photobleaching of bR became significant above 30 °C at which DMPC membrane is in the lipid crystalline phase. An interesting question arises from this previous result: At which temperature the photobleaching occur for reconstituted bR in dipalmitoylphosphatidylcholine (DPPC) bilayer whose main transition is 42 °C. In the photobleaching measurement, suspensions of reconstituted bR membrane which were reconstituted in DMPC and DPPC membranes were incubated at high temperature for an hour under illumination or in the dark and then quenched to about 4 °C. The ratio of native bR after incubation to total bR was estimated by the absorbance at 560 nm. The photobleaching of bR in DPPC began at about 30 °C and the temperature dependence of the ratio of photobleached bR in DPPC membrane was the same as that in DMPC membrane. However, small but significant difference was observed between the two types of reconstituted bR below 30 °C. The onset temperature of the photobleaching of reconstituted bR membrane in DMPC bilayer was actually about 20 °C. We will discuss the relationship between the onset temperature of the photobleaching and the three types of phase transitions of lipid bilayer membrane: main, pre- and sub-transitions.

3P-086 バクテリオロドプシンに対するフォースカーブ測定のための細胞質側と細胞外側の識別

Identification of cytoplasmic and extracellular side of bacteriorhodopsin for force spectroscopy

Yosuke Higashi (1), Satoshi Ozaki (1), Naoya Watanabe (2), Yasunori Yokoyama (1), Masashi Sonoyama (1), Shigeki Mitaku (1) ((1) Graduate School of Engineering, Nagoya University; (2) SII NanoTechnology Inc.)

Recently, force spectroscopy is extensively studied for evaluating intra- and inter-molecular interaction. Bacteriorhodopsin is one of typical membrane protein which has been used for the experiments of force spectroscopy.

We are developing experimental and computational methods for estimating the intra-/inter-interaction in membrane proteins by the force spectroscopy. On one hand, amino acid chemical potentials in membrane and in water are considered in this simulation. The simulation showed the force curves from the cytoplasmic side and from extracellular side. In the present study, we have established a method to identify the cytoplasmic and extracellular sides for force spectroscopy.

AFM is SPA400(SII nanotechnology). Constant of spring of cantilever is 0.08 N/m, and tip diameter is not over 20 nm. Measurements were carried out at the room temperature, and in darkness. Purple membrane suspension which controlled pH was adsorbed onto freshly cleaved mica. Half an hour later, rinsed once with buffer solution. Membranes were observed in buffer, and obtained force spectrum.

The difference of the images of cytoplasmic and extracellular sides is significant in air but insignificant in buffer solution. But we first took the images of bacteriorhodopsin in buffer solution and then those in air. Comparing the images, we found the global characterization of purple membrane in the buffer solution. We used this result to obtain force spectrum from cytoplasmic and extracellular side by AFM.

We will show the comparison between the experimental force spectra and the computational simulation.

3P-087 大腸菌機械受容チャネル MscL のメカノゲーティングにおける水の浸潤と透過の役割

Role of Water Penetration and Permeation in Mechano-Gating of the *E-coli* Mechanosensitive Channel MscL

Yasuyuki Sawada (1), Masaki Murase (2), Masahiro Sokabe (1) ((1) Dept. Physiol. Nagoya Univ. Grad. Sch. Med.; (2) ICORP/SORST Cell Mechanosensing, JST)

The bacterial mechanosensitive channel of large conductance MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer α -helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the

membrane. Although several models for the opening process have been proposed based on molecular dynamics (MD) simulations, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. We performed MD simulations for the mechano-gating of MscL embedded in the lipid bilayer. Upon tension generation in the bilayer, Phe78 in the outer helix was dragged by lipids, leading to a tilting of the helices. Among amino acids in the outer helix facing the bilayer, Phe78 at the water-lipid interface showed the strongest interaction with lipids, thus may work as a major tension sensor. Neighboring inner helices cross each other in the inner leaflet, forming the most constricted part of the pore. In the closed state of MscL, Leu19 and Val23 in the constricted part form a stable hydrophobic environment. As tension increases, the crossings move toward the cytoplasm associated with an expansion of the constricted part and the hydrophobic environment was broken followed by water penetration and permeation. It seemed that water penetration and permeation accelerated the pore opening probably by decreasing the hydrophobic interaction between Leu19 and Val23. To support this idea, we performed MD simulations of the GOF mutant G22N with almost the same pore size, and found that G22N mutant MscL permeated water molecules without tension in the bilayer.

3P-088 FGF23 分泌における ppGalNAc-T3 の役割 Role of Polypeptide GalNAc-transferase T3 in FGF23 secretion

Kentaro Kato (1), Charlotte Jeanneau (2), Imran Dar (2), Mads Agervig Tarp (2), Anna Benet-Pagès (3), Eric Paul Bennett (4), Ulla Mandel (4), Tim M. Strom (3), Henrik Clausen (2) ((1) Department of Medical Biochemistry and Genetics, University of Copenhagen; Recently moved to Graduate School of Advance Life Science, Hokkaido University; (2) Department of Medical Biochemistry and Genetics, University of Copenhagen; (3) GSF National Research Center; (4) Faculty of Health Sciences, School of Dentistry, University of Copenhagen)

Mutations in the glycosyltransferase, polypeptide GalNAc-T3 gene, involved in initiation of O-glycosylation, were recently identified as a cause of the rare autosomal recessive metabolic disorder familial tumoral calcinosis (FTC)(OMIM 211900). FTC is associated with hyperphosphatemia and massive ectopic calcifications. Here, we demonstrate that the secretion of the phosphaturic factor FGF23 requires O-glycosylation, and that GalNAc-T3 selectively directs O-glycosylation in a subtilisin-like proprotein convertase recognition sequence motif, which blocks processing of FGF23. Furthermore, this O-glycosylation is depending on the lectin domain of GalNAc-T3.

The study suggests a novel posttranslational regulatory model of FGF23 involving competing O-glycosylation and protease processing to produce intact FGF23.

3P-089 Visualization of the assembly and disassembly of *S. cerevisiae* Rad51 on duplex DNA in real-time

Ichiro Amitani (1), Amitabh V. Nimonkar (1), Ronald J. Baskin (1), Stephen C. Kowalczykowski (1) ((1) University of California, Davis)

DNA double-strand breaks (DSB) are the most dangerous type of DNA damage. Homologous recombination (HR) is the most reliable method to repair DSBs. The process of HR is separated into three phases, in which a RecA-like DNA strand exchange protein plays a central role. First, the end of broken DNA is processed to generate 3'-ended single-strand DNA (ssDNA) tail to which the DNA strand exchange protein binds. Second, the protein-DNA complex searches homology on a donor double-strand DNA (dsDNA) and catalyzes the pairing and exchange of DNA strands. Finally, the repaired DNA molecules are resolved.

In *S. cerevisiae*, HR is catalyzed by a RecA-like protein, Rad51. Rad51 forms a helical filament in an ATP dependent manner. In the filament, Rad51 occupies 3-4 nucleotides and stretches DNA to 150% of its B-form length. Interestingly, Rad51 binds to dsDNA with a similar affinity as for ssDNA, even though the functional form of Rad51 requires assembly on ssDNA. Since the Rad51 filament formation on dsDNA inhibits HR, the turnover of Rad51 on dsDNA is stimulated by a dsDNA translocase, Rad54.

In this study, we use single molecule fluorescence microscopy to observe the assembly and disassembly of Rad51 filament on dsDNA in real-time. We show that the Rad51 filament is stable in the presence of nucleotide cofactors and that Rad51 can hydrolyze multiple ATP molecules while remaining bound to DNA.

3P-090 バクテリオファージ Mu の標的免疫反応の光学顕微鏡下での直接観察 Direct Observation of Bacteriophage Mu Transposition Target Immunity

Yong-Woon Han (1), Kiyoshi Mizuuchi (2) ((1) Institute for Integrated Cell-Material Sciences, Kyoto University; (2) National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, USA)

DNA transposons integrate into the chromosome of their host organisms at many sites with limited target sequence specificity. In the absence of a mechanism to avoid insertion into itself, transposons would risk self-destruction. Some transposons can avoid this problem, and transposition immunity of phage Mu is

one example. MuA transposase and MuB ATP-dependent DNA-binding protein participate in this process. MuB-bound DNA is an efficient target for Mu insertion. MuA bound to the Mu DNA ends stimulates the MuB ATPase and dissociates MuB from its neighborhood, making the region near Mu ends a poor target. The stable MuA tetramer in the Mu transpososome efficiently disassembles MuB from DNA by forming DNA-loops between the transpososome and the MuB bound DNA site. We show that the average observable DNA loop size expands as the duration in which the DNA Brownian motion for loop formation is extended. The transpososome dissociate MuB molecules first from nearby DNA by forming smaller DNA loops and as the nearby MuB becomes scarce, finds MuB oligomers further away to form larger DNA loops. We propose a model in which several rounds of DNA loop formation/dissociation cycles results in translocation of the transpososome away from the transposon ends prior to insertion into distant target DNA sites.

3P-091 SUMO 化による転写調節因子 heat shock factor 2 の DNA 結合阻害の分子機構 Molecular basis for SUMOylation-dependent regulation of DNA binding activity of Heat Shock Factor 2

Mariko Ariyoshi (1), Yukihiko Tateishi (1), Ryuji Igarashi (1), Hideyuki Hara (2), Kenji Mizuguchi (3), Hidehito Tochio (1), Masahiro Shirakawa (1) ((1) Graduate School of Engineering, Kyoto University; (2) ESR Division, Bruker Biospin K.K.; (3) National Institute of Biomedical Innovation)

The heat shock response is a cellular defense against environmental and physiological stresses, such as heat, heavy metals, oxygen radicals and viral or bacterial infections. Eukaryotic cells maintain homeostasis through elevating the expression of heat shock proteins (HSPs), which function as molecular chaperones, in response to various stress stimuli. The heat shock response is also evoked during non-stress-induced processes, such as early development and cellular differentiation in mammals. Heat shock factor 2 (HSF2) is a member of a vertebrate transcription factor family for genes of HSPs and is involved in the regulation of development and cellular differentiation. The DNA binding property of HSF2 is modulated by the post-translational modification of a specific lysine residue in its DNA binding domain by small ubiquitin-like modifier (SUMO). However the consequences of SUMOylation and its underlying molecular mechanism remain unclear. Here we show the inhibitory effect of SUMOylation on the interaction between HSF2 and DNA based on biochemical analysis using isolated recombinant HSF2. NMR study of the SUMOylated DNA binding domain of HSF2 indicates that the SUMO moiety is flexible with respect to the DNA binding domain and has neither a noncovalent interface with nor a structural effect on the domain. Combined with data from double electron-electron resonance and paramagnetic NMR relaxation enhancement experiments, these results suggest that SUMO attachment negatively modulates the formation of the protein-DNA complex through a randomly distributed steric interference.

3P-092 部位特異的変異体を用いた bHLH 因子の解析 Analyses of a bHLH transcription factor using site-directed mutants

Keigo Furuya (1), Koji Hasegawa (1), Osamu Hisatomi (1) ((1) Osaka University)

Basic helix-loop-helix (bHLH) transcription factors play a role in regulation of transcription. They form a homo-dimer or a hetero-dimer with other bHLH factors, and that control the transcription of genes by binding to a specific DNA sequence, E-box. However, the dynamic processes of their dimerization and DNA binding remains to be elucidated. To understand these processes, we expressed GFP-fusion protein (HEB-GFP) of a bHLH transcription factor, HEB protein (Hela E-box-binding protein) that expressed ubiquitously in most cell types of many vertebrates. When HEB-GFP was mixed with the Cy5-labeled oligonucleotides containing the E-box sequence (Cy5-Eoligo), the fluorescence resonance energy transfer (FRET) was observed, suggesting that HEB-GFP bound to Cy5-Eoligo similar to native HEB. However, HEB-GFP formed dimers even in the absence of Eoligo at low temperature unlike recombinant HEB. Dissociation constant (Kd) obtained from FRET analysis was about 9 nM for Eoligo, which shows a good agreement with those obtained from other methods (for example gel-shift assay). Then, we prepared site-directed mutants of HEB-GFP in which the 9th amino acid, a glutamic acid (Eb9), in basic region of HEB were replaced into glycine, glutamine and aspartic acid. These mutant HEB-GFPs bound Eoligo with higher Kd (19-24 nM). It was suggested that Eb9 plays a crucial role for recognition of E-box sequence.

3P-093 Leucyl-tRNA 合成酵素における酵素機能発現の計算科学的解析 Computational analyses of expression mechanisms of enzymatic functions in leucyl-tRNA synthetase

Yohsuke Hagiwara (1), Osamu Nureki (2), Masaru Tateno (3) ((1) University of Tsukuba; (2) University of Tokyo; (3) University of Tsukuba)

Aminoacyl-tRNA synthetases (aaRSs) catalyze the attachment of their cognate amino acid to the 3'-end of the specific tRNAs; their strict discrimination of the cognate amino acids from non-cognate ones is crucial for the fidelity of translation. However, in some cases, e.g., the leucine system, due to existence of